Calcium Release Activated Calcium Channels

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Structural Modeling of Hexameric and Tetrameric Ion Conduction Pathways of Orai1 Channel
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Ca^{2+} release-activated Ca^{2+} (CRAC) channels mediate Ca^{2+} entry in response to store depletion in a variety of cell types. Endogenous CRAC channels in mammals are formed by a homomeric assembly of Orai1 proteins. Earlier functional studies involving chemical cross-linking of different numbers of wild-type Orai1 subunits, electrophysiological recordings and single molecule fluorescence analysis techniques as well as high-resolution electron microscopic examination of purified Orai proteins strongly indicated that functional Orai channels were most likely tetramers. In contrast, study of crystals containing holo-dOrai channel complexes from *Drosophila melanogaster*, revealed that a single channel complex contains six dOrai subunits. The hexameric CRAC channel stoichiometry was further supported by cross-linking and size-exclusion chromatography studies of the *Drosophila* Orai. Recent functional study revealed that expression of concatenated hexameric Orai1 channel produces a non-selective cation channel with biophysical properties essentially different from those of endogenous CRAC channels or channels formed by expressed concatenated tetrameric Orai1 proteins. Thus, the studies reported are far from conclusive but they do indicate the need for further investigation of Orai1 channel stoichiometry. Accordingly, we generated structural models of Orai1 ion conduction pathway formed by tetrameric or hexameric assembly of Orai1 pore-lining TM1 segments using Rosetta fold and dock protocol. Based on available experimental data we constrained proximity of several key residues lining Orai1 ion conduction pathway during simulations. Among the lowest energy and most frequently sampled conformations of Orai TM1 hexamers generated by Rosetta, we identified structural models that were in close agreement with Orai structure in the transmembrane region proposed based on crystallographic data analysis. Our preliminary models of Orai TM1 tetramers suggest alternative structural topology forming ion conduction pathway, which may account for the differences in ionic selectivity of hexameric and tetrameric Orai1 channels.

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Complex Function of Stim1 in the Activation of Store-Independent Orai Channels
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Orai proteins contribute to Ca^{2+} entry pathways through store-dependent, Ca^{2+} release-activated Ca^{2+} (CRAC) channels (Orai1), and store-independent, arachidonic acid (AA)-regulated Ca^{2+} (ARC) or LeukotrieneC4-regulated Ca^{2+} (LRC) channels (Orai1 and Orai3). Remarkably, although activated by fundamentally different mechanisms, both CRAC and ARC/LRC channels share a requirement for STIM1 expression. To date the role of endoplasmic reticulum-resident STIM1 (ER-STIM1) in the activation of CRAC channels is well appreciated. There is a minor pool of STIM1 at the plasma membrane (PM-STIM1) that was shown to be necessary for ARC current activation in HEK293 cells. Using pharmacological tools targeting AA synthesis and metabolism, Ca^{2+} imaging, whole-cell and perforated patch clamp electrophysiological recordings we demonstrate that both Orai1 and Orai3 are required for ARC and LRC current activation in both primary vascular smooth muscle cells (VSMCs) and HEK293 cells. Surprisingly, while PM-STIM1 is required for ARC and LRC current activation under whole cell patch clamp recordings in both cell types, ER-STIM1 is sufficient for both ARC and LRC channel activation when intact cells are considered. These results are first to demonstrate ARC channel function in primary VSMCs, highlight the complexity of STIM1 regulation of store-independent Orai channels and demonstrate that ARC and LRC currents are mediated by the same channels.

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Distinct Orai-Coupling Domains in Stim1 and Stim2 Define the Orai-Activating Site
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The ER membrane-spanning STIM1 protein is a finely-tuned sensor of ER luminal Ca^{2+}. Small changes in ER Ca^{2+} induce STIM1 to undergo an intricate self-triggering process, causing it to translocate into ER-PM junctions where it couples with and activates the highly Ca^{2+}-selective family of Orai channels in the PM. The entering Ca^{2+} sustains Ca^{2+} oscillations, maintains Ca^{2+} homeostasis, and provides crucial long-term Ca^{2+} signals in many cell types which control gene expression and cellular growth. Similar in structure and also widely expressed among cells, the little-studied STIM2 protein is reported to differ subtly from STIM1 in its N-terminal domain, affecting luminal Ca^{2+}-sensitivity and the rate of unfolding and self-activation. The STIM1 cytoplasmic C-terminus contains the STIM-Orai activating region (SOAR) which has been structurally resolved. While the corresponding SOAR sequence in STIM2 is highly conserved, we reveal it has a profoundly diminished interaction with and ability to gate Orai1 channels. We narrowed this distinction in Orai1 activation to a small sequence in SOAR, within which substitution of a single phenylalanine in STIM1 with leucine in STIM2 confers a severe decrease in Orai1 channel-gating efficacy. This residue is strategically positioned at the structural apex of the SOAR domain. Modification of this single residue within the intact STIM1 protein reveals its pivotal role in both interaction with and gating of the Orai1 channel. The results not only pinpoint a crucial locus of STIM-Orai coupling but also reveal a physiologically profound distinction between STIM1 and STIM2.

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Stim1 Cytosolic Coiled-Coil Interactions in the Resting and Activated State
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STIM1 and Orai1 are key components of the Ca^{2+}-release activated Ca^{2+} (CRAC) current that plays an important role in T cell activation as well as mast cell degranulation. Activation of the CRAC channel forming subunit Orai1 occurs via a physical interaction with the ER transmembrane Ca^{2+} sensor protein STIM1 when ER Ca^{2+} stores are depleted. This CRAC channel activation process is accompanied by a conformational change of STIM1 into an extended conformation together with puncta formation. Consequently SOAR/CAD is exposed during this process and drives oligomerization, probably by interhelical rearrangements between the three cytosolic STIM1 coiled-coil (CC) domains. Here we focused on intra- and inter-molecular interactions specifically between CC2/CC3 and the three α-helices comprising CC1. In an attempt to differentially examine and map possible interactions between these three α-helices and the SOAR/CAD comprising CC2 and CC3, a system termed ''FRET-based Interactions in Restricted Environments (FIRE)'' was developed. Furthermore, single point mutations were introduced into these helical fragments to eliminate or strengthen their interactions. In extension of these results, we additionally inserted point mutations and deletions into full length STIM1 and the YFP-OASF-CFP-FRET sensor for further functional analysis by patch-clamp and FRET measurements. Our results revealed new insights into the mechanism linking STIM1 oligomerization to the differential interactions of specific α-helices of CC1 with CAD/SOAR and allowed us to delineate a model describing STIM1 activation following store depletion. (supported by Austrian Science Fund (FWF): P22747 to R.S., P22565 and P25172 to C.R.)

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Stim1 Binds to Pairs of Orai1 Subunits to Open the Crac Channel
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CRAC channels are activated by binding of the ER Ca^{2+}-sensor STIM1 to the cytoplasmic C-terminus of the channel subunit Orai1. The crystal structure of *Drosophila* Orai describes a trimer of Orai dimers in which each C-terminus forms an antiparallel coiled-coil with its neighbor. This unexpected arrangement raises the question of whether pairs of C-termini cooperate to bind STIM1 and thereby function as a unit, or whether single C-termini act independently to bind STIM1 and activate Orai1.
We assayed binding by E-FRET between CFP-labeled Orai1 tandem dimers and YFP-labeled STIM1 CRAC activation domain (aa#342-448) expressed in HEK cells. FRET indicated strong binding to normal (WT-WT) dimers and no binding to L273D-L273D dimers, as expected (the C-terminal L273D mutation prevents STIM1 binding). Dimers with one C-terminus deleted (WT-CT) had weak FRET, indicating that STIM1 binds weakly to the 3 monomeric C-termini in the assembled channel. However, WT-L273D heterodimers produced significantly higher FRET, showing that the ''non-binding'' L273D C-terminus contributes to STIM1 binding when paired with a WT C-terminus. These results suggest that STIM1 binds to pairs of Orai1 C-termini in the native channel. To quantify the effects of L273D on Orai1 activation, we generated hexameric concatemers with L273D mutations in subunits 1, 1+3, or 1+3+5, producing channels with one, two, or three heterodimers, respectively. When co-expressed with saturating levels of STIM1, WT concatemers generated normal CRAC-like currents. L273D reduced the current magnitude in a highly nonlinear manner; each heterodimer reduced activity by ~2/3, while